Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.13 (1): 65-75; March 2024 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v13-i1-535

Niacin ester derivative of Oleanolic acid as a potential antidiabetic agent: an *in silico* and *in vitro* study

[1]***Victor U. Anah,** [2]**Samuel J. Offor,** [1]**Aniekan S. Ebong,** [2]**Nkechi J. Onyeukwu,** [3]**Romanus A. Umoh,** [1]**Olorunfemi A. Eseyin**

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria.

[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria.

[3]Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

ABSTRACT

Background: Oleanolic acid (OA) has been found to exert beneficial effects against Type 2 Diabetes mellitus and metabolic syndrome. This study aimed at designing derivatives of OA and evaluating their binding affinities to protein targets implicated in diabetes. Synthesis, physicochemical and *in vitro* studies were carried out on a promising ligand – niacin derivative (ND).

Method: Eight derivatives of OA and two control drugs (metformin and acarbose) were designed with ChemDraw ultra. The eight targets were: α-amylase (AAM); Protein Tyrosine Phosphatase-1B (PTP1B); Dipeptidyl peptidase (DPP4); α-glucosidase (AGCS); Glycogen synthase kinases-3β (GSK3); Fructose-1,6-diphosphatase (F1,6DP); Peroxisome proliferator-activated receptor-γ (PPARG) and Glucokinase (GLK). These were downloaded from the Protein data bank. Ligands and targets were converted to pdbqt format using Autodock tools. Molecular docking was done using Autodock Vina. Discovery Studio was used to analyze ligand-protein binding interactions. Synthesis of ND was done based on acyl chloride nucleophilic substitution method. *In vitro* study was done using α-amylase inhibition and glucose uptake by yeast cells methods.

Results: *In silico* study showed that ND had a better binding affinity on seven targets over OA. (AAM, DPP4, PPARG, PTB1B, F16DP, GSK and GLK). The *in vitro* α-amylase inhibition values (IC_{50}) for Acarbose, OA, and ND were 48.21±0.56, 26.40±0.32, and 24.25±0.52 µg/mL, respectively. For % glucose uptake by yeast cells OA, ND and Glibenclamide gave 80.2, 88.5 and 72.6, respectively).

Conclusion: Higher binding affinities, low IC_{50} and higher glucose uptake by yeast cells observed in niacin derivative (ND) showed that it is a potential antidiabetic compound.

Keywords: Diabetes mellitus, *in silico*, *in vitro*, niacin, oleanolic acid.

1. INTRODUCTION

The term, 'diabetes' describes a group of metabolic disorders characterized and identified by the presence of hyperglycaemia in the absence of treatment. Its various heterogeneous aetio-pathology include defects in insulin secretion, insulin action, or both, and disturbances of carbohydrate, fat and protein metabolism. Diabetes is found in

* **Corresponding author: Email: progress.obazelu@uniben.edu; Phone: +2348056733255**

every population in the world and in all regions, including low- and middle-income countries where it is often accompanied by other diseases [1]. It is considered the third most common chronic and non-contagious disease after cancer and cardiovascular diseases [2]. Current therapies applied for diabetic treatment have a lot of limitations. These limitations, the high cost of treating diabetes and its complications and the increased prevalence of diabetes globally point to the need for efficient, affordable and more effective alternative treatment. Over 1,200 plant species are reported in ethno-medicine for treating diabetes and this gives an important lead for the identification and synthesis of novel antidiabetic drugs [3]. One of such promising compounds from ethno-medicine origin for diabetes treatment is Oleanolic acid [OA]. Throughout complex and multifactorial mechanisms, OA exerts beneficial effects against diabetes and metabolic syndrome. It improves insulin response, preserves functionality and survival of β-cells, and protects against diabetes complications. OA may directly modulate enzymes connected to insulin biosynthesis, secretion, and signaling [4,5]. However, OA's major contributions appear to be derived from the interaction with important transduction pathways, and many of its effects are consistently related to activation of the transcription factor, Nrf2. Doing that, OA induces the expression of antioxidant enzymes and phase II response genes, blocks necrotic factor (NF-Kb), and represses the polyol pathway, AGEs production, and hyperlipidemia [6]. Molecular docking is a compelling framework for comprehending drug-bimolecular interaction, which is useful for both mechanistic research and rational drug design and discovery [7]. Some derivatives of OA had been synthesized and tested for antidiabetic potency with some promising outcome especially in 3-ethoxy and 3-cinnamoyl derivatives [8,9]. Other derivatives have been synthesized and tested for other pharmacological activities such as; antimicrobial, antiplasmodial and anticancer activities [10,11]. The aim of this study was to synthesize a novel OA derivative with improved antidiabetic activity when tested *in vitro* over OA.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Perkin Elmer Lambda 25 UV–VIS spectrophotometer was used for UV-Vis Spectroscopy studies.

2.1.2 Chemical Materials

Oleanolic acid was obtained from Biosciences, New York while nicotinic acid and other reagents were obtained from Sigma-Aldrich, UK.

2.2 Methods

2.2.1 In silico docking studies on oleanolic acid derivatives and protein targets

The method of Eseyin *et al.*, [12] was followed. Oleanolic acid and derivatives were designed with ChemDraw Ultra 12.0 version (CambridgeSoft Corporation, USA) and saved in SDF format (figure 1) and (Table 1). The eight targets implicated in diabetes included: α-amylase (AAM-1B2Y); Protein Tyrosine Phosphatase 1B (PTP1B-1QIM); Dipeptidyl peptidase (DPP4-1JZE); α-glucosidase (AGCS-7K9n); Glycogen synthase kinases 3β (GSK3-7s6u); Fructose-1,6-diphosphatase (F1,6DP-3OHI); Peroxisome proliferator-activated receptor gamma (PPARG-8Dk4) and Glucokinase (GLUCK-1Q18). They were downloaded in PDB format from the Protein data bank (http://www.rcsb.org/pdb/home/home.do). Ligands and targets were converted to pdbqt format using Autodock tools [13]. Molecular docking of the ligands with each of the target proteins was done using Autodock Vina (http://vina.scripps.edu/), to obtain their respective binding affinity. The grid box parameters were generated from autodock vina for each target and shown in Table 2. Discovery Studio (Dassault Systèmes was used to analyze ligand-protein binding interactions.

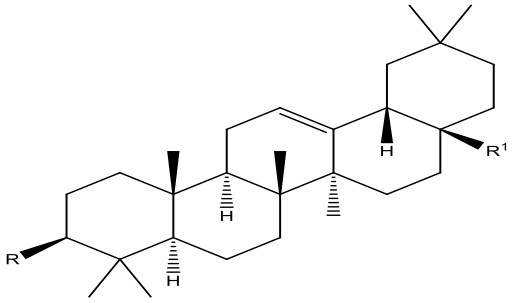


Figure 1: Structure of Oleanolic acid and derivatives

Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.13 (1): 65-75; March 2024 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v13-i1-535

Table 1: Oleanolic acid and derivatives designed and used in this study

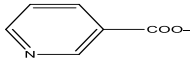
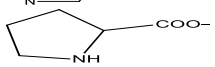
S/N	Ligand	R	R^1
1	Oleanolic acid	OH	COOH
2	3-Oleanylethanoate	CH_3COO-	COOH
3	3-Oleanyl-n-valerate	C_4H_9COO-	COOH
4	3-Oleanlylniacinate (ND)		COOH
5	3-Oleanlylprolinate		COOH
6	28-Butyloleanate	OH	$COOC_4H_9$
7	28-Propyloleanate	OH	$COOC_3H_7$
8	28-Methyloleanate	OH	$COOCH_3$
9	Acarbose	-	-
10	Metformin	-	-

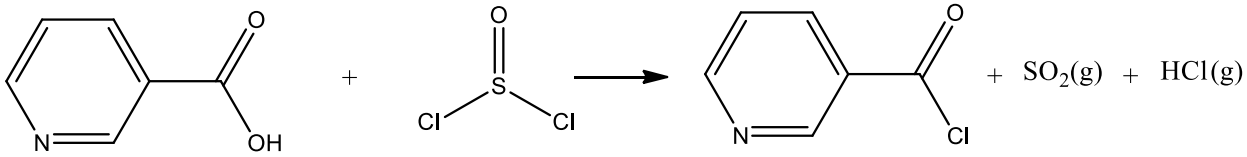
Table 2: Grid box parameters

S/N	Target	center_x	center_y	center_z	size_x	size_y	size_z
1	AGCS	22.992	7.307	-24.059	78	84	86
2	GLK	21.037	20.308	26.953	62	70	84
3	AAM	21.593	17.109	24...498	74	66	92
4	PPARG	-23.088	-12.005	13.770	76	70	82
5	DPP4	48.645	59.931	31.936	88	86	88
6	PTP1B	36.889	29.781	32.546	82	86	82
7	F16BP	9.459	-3.799	16.560	76	68	74
8	GSK3	53.580	23.431	42.148	84	74	82

AGCS–alpha-glucosidase; GLK-glucokinase; AAM–alpha-amylase; PPARG–peroxisome proliferator-activated receptor-γ; DPP4–dipeptidyl peptidase-IV; PTP1B – protein tyrosine phosphatase-1B; F16BP – fructose-1,6-diphosphatase; GSK3 – glycogen synthase kinases-3β.

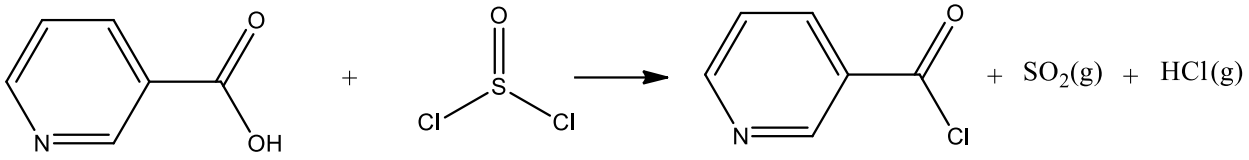
2.2.2 Synthesis of Niacin ester derivative of OA (Oleanyl niacinate) (ND)

Eight grammes (8.0 g) of niacin (nicotinic acid) acid was measured into a 250-mL round bottom flask fitted to a reflux apparatus. Thionyl chloride (75 mL) was introduced into the flask and a few clean pebbles added to act as anti-bump. Refluxing was allowed to run for four hours in the hood. Excess thionyl chloride was distilled out (boiling point = 79°C) while formed SO_2 and HCl were expelled as gases in the hood. The reaction is shown below;



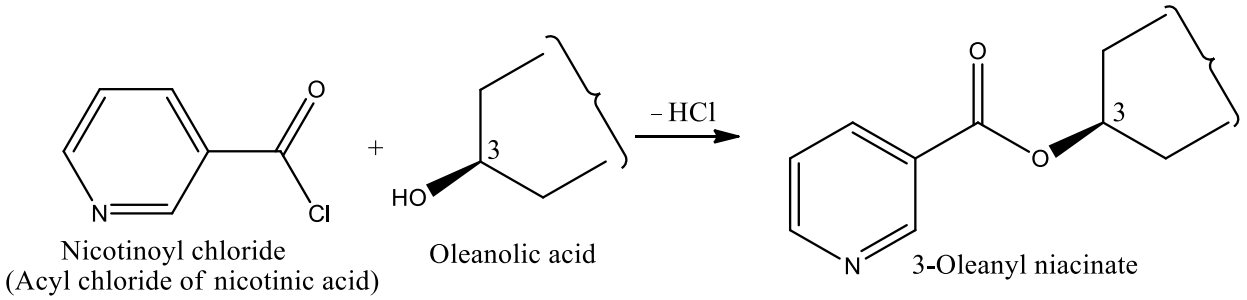
Nicotinic acid Thionyl chloride Acyl chloride of nicotinic acid (Nicotinoyl chloride)

Acyl chlorides undergo nucleophilic substitution as Cl are expelled as Cl^- or HCl. The formed nicotinoyl chloride was allowed to cool. Dichloromethane (100 mL) was introduced into the flask with swirling. Oleanolic acid (5.0 g) was introduced and stirred till it dissolved. Dimethyl sulphate, $(CH_3)_2SO_4$ in ethanolic solution of NaOH (5.0 mL) was added to block reactivity at C-28(COOH) of OA. Pyridine (2 mL), an organic base was added as catalyst - Schotten Baumann technique [14] and the contents of flask gently stirred. Mild refluxing was carried out at temperature of 30°C in a water bath for ten hours till a product was formed through TLC monitoring. The equation of reaction is shown below.



Nicotinic acid | Thionyl chloride | Acyl chloride of nicotinic acid (Nicotinoyl chloride)

The obtained product was purified using column chromatography (CC) packed with silica gel (60-120 mesh size) prepared by wet slurry method and eluted with chloroform ($CHCl_3$) in a hood using gradient elution. The eluent with the desired Rf value was evaporated and obtained product recrystallized in DCM. The reaction is shown below.



Nicotinoyl chloride (Acyl chloride of nicotinic acid) | Oleanolic acid | 3-Oleanyl niacinate

2.2.3 Physicochemical properties of 3-oleanyl niacinate (ND) and OA

Rf value: Using a solvent system of n-hexane and ethyl acetate (3:2), a TLC is run on precoated silica gel plate. Developed plate was sprayed with 10% ethanolic solution of H_2SO_4 and dried for about 5 minutes in oven at 110°C. The spots were noted and measured.

Solubility Profile: A little quantity of OA and ND were put inside test tubes separately. About 3 mL of different solvents were added and their solubility observed.

UV-Vis Spectroscopy: Samples were scanned in UV-Vis spectrophotometry. A 1 mg/ml solution of OA and ND were prepared in n-hexane:Acetone (2:1). Their peak absorbances and λmax were recorded.

Presence of carboxylic acid group: Two (2.0) mg of OA and ND were separately put inside a test tube. Paper soaked in $Ca(OH)_2$ was placed near the mouth of the test tube. $NaHCO_3$ solution (5 mL) was gently introduced into the test tube and shaken. Turbid milky white colour shows the presence of -COOH as CO_2 was evolved.

2.2.4 In vitro antidiabetic studies on 3-Oleanyl niacinate (ND)

2.2.4.1 Alpha-amylase inhibition method

Inhibitors of alpha-amylase delay the breaking down of carbohydrates and diminish the postprandial blood glucose excursion. The method of Hansawasdi *et al.*, [15] was carried out following standard protocol with slight modifications. Starch azure (2 mg) was suspended in 0.2 mL of 0.5M Tris–HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ (substrate solution). The tubes containing substrate solution was boiled for 5 minutes and then pre-incubated at 37°C for 5 minutes. The following strength of oleanolic acid/derivative in ethanol were prepared: 20, 40, 60, 80, and 100 µg/mL. Then, 0.2 ml each of these preparations were added to the tube containing the substrate solution. In addition, 0.1 ml of porcine pancreatic amylase in Tris–HCl buffer (2 units/mL) was added to the tube containing oleanolic acid/derivative. The reaction was carried out at 37°C for 10 minutes and stopped by adding 0.5 mL of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 minutes at 4°C. The absorbance of resulting supernatant was measured at 595 nm using spectrophotometer. Acarbose, a known α-amylase inhibitor was used as standard drug. The experiment was repeated thrice. The α-amylase inhibitory activity was calculated by using following formula:

$$\text{Alpha} - \text{amylase inhibitory activity} = (\text{Ac})^{+} - (\text{Ac})^{-} - \frac{(\text{As} - \text{Ab})100}{(\text{Ac})^{+} - (\text{Ac})^{-}}$$

[where Ac+, Ac−, As, and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme), and a blank (a test sample without enzyme), respectively. The concentration of acarbose and drug compounds required to inhibit 50% of α-amylase activity under these conditions was defined as the IC_{50} value. The α-amylase inhibitory activities of oleanolic acid/derivative and acarbose were calculated and their IC_{50} values determined.

Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.13 (1): 65-75; March 2024 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v13-i1-535

2.2.4.2 Glucose uptake by Yeast cells method

The method of Cirillo was followed in this procedure with minor modification [16]. One milliliter (1.0 mL) each of glucose solution at various concentrations (20-100 µg/mL) was transferred to various test tubes containing 4.0 mL of 10 % v/v suspension of yeast solution prepared by repeatedly washing yeast with distilled water and centrifuging at 3,000 rpm for five minutes. The solution was incubated in a dark cupboard for one hour. The test solution (1.0 mL) was transferred to the test tubes containing glucose and yeast solution mixture, vortexed and further incubated at 37°C for 60 minutes. The percentages increase in glucose uptake (% U) by yeast cells were calculated using the equation below;

$$\%\,U = \frac{(As - Ac\,)100}{As}$$

Where Ac is the absorbance of the control which does not contain the sample and As is the absorbance of the solution in the presence of the different samples and U is Uptake. Glibenclamide was used as standard drug.

2.3 Statistical Analysis

The results of this research were expressed as Mean ± SD and were analyzed by one-way Analysis of Variance (ANOVA) using GraphPad Prism version 5.03. Values with $p < 0.05$ were considered significant.

3. RESULTS

3.1 In silico studies

Binding affinity (kcal/mol) of the ligands and reference compounds are presented in table 3.

Table 3: Binding affinity (kcal/mol) of the ligands and reference compounds obtained from Autodock vina.

S/N	LIGAND	AGCS	GLK	AAM	PPARG	DPP4	PTP1B	F1,6BP	GSK3
1	Oleanolic acid	-8.6	-8.5	-8.0	-7.4	-8.4	-8.6	-7.6	-8.3
2	3-Oleanlylethanoate	-7.6	-6.0	-8.5	-7.5	-8.7	-7.0	-6.8	-8.2
3	3-Oleanyl-n-valerate	-8.2	-5.9	-8.6	-7.1	-8.4	-7.2	-6.7	-8.6
4	3-Oleanlylniacinate (ND)	-8.5	-9.1	-9.0	-9.0	-9.3	-9.3	-8.8	-8.9
5	3-Oleanlylprolinate	-8.4	-6.7	-8.7	-8.0	-8.1	-7.8	-7.7	-8.1
6	28-Butyloleanate	-6.8	-5.6	-7.8	-7.5	-8.4	-7.1	-6.3	-8.5
7	28-Propyloleanate	-7.7	-6.2	-8.2	-7.4	-8.3	-7.2	-6.8	-8.7
8	28-Methyloleanate	-7.3	-6.2	-8.5	-8.0	-8.8	-7.9	-7.3	-8.3
9	Acarbose	-8.0	-6.4	-8.2	-8.2	-8.6	-7.5	-7.0	-8.6
10	Metformin	-4.8	-5.1	-5.2	-5.0	-5.0	-4.8	-3.9	-4.8

3.2 Result of Protein-binding interactions

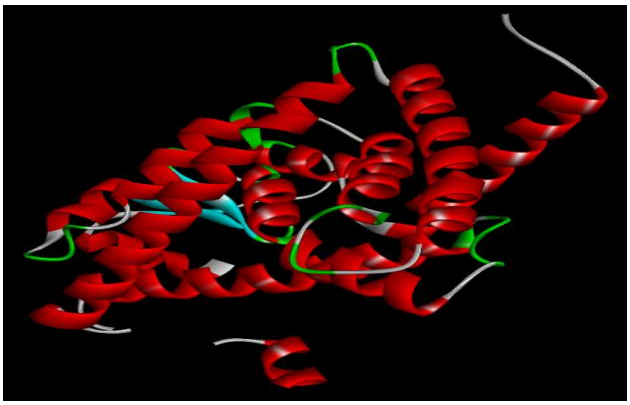


Figure 2a: Peroxisome proliferator-activated receptor-γ, PPARG (8Dk4)

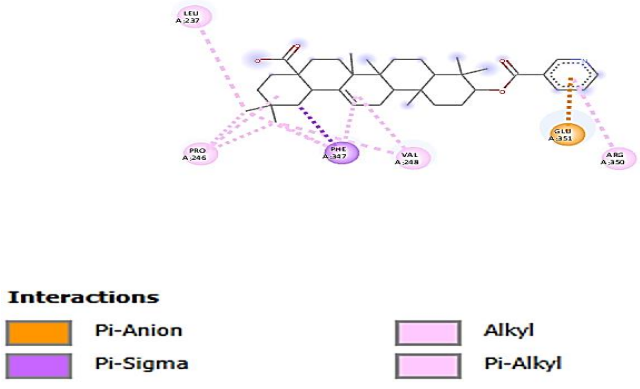


Figure 2(b): Interaction of PPARG-8Dk4 with ND

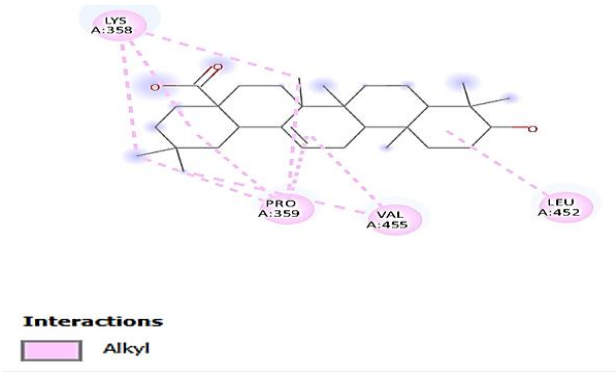


Figure 2(c): Interaction of PPARG-8Dk4 with OA

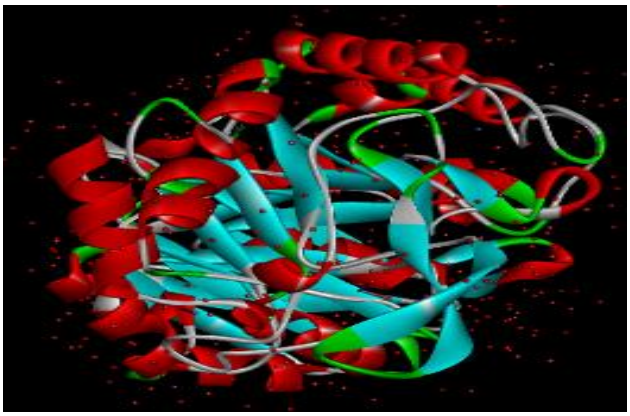


Figure 2(d): α-amylase, AAM (1B2Y)

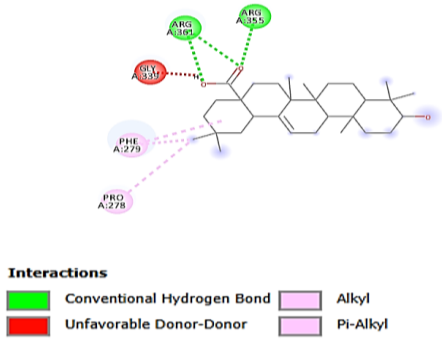


Figure 2(e): Interaction of α-amylase (AAM-1B2Y) with OA

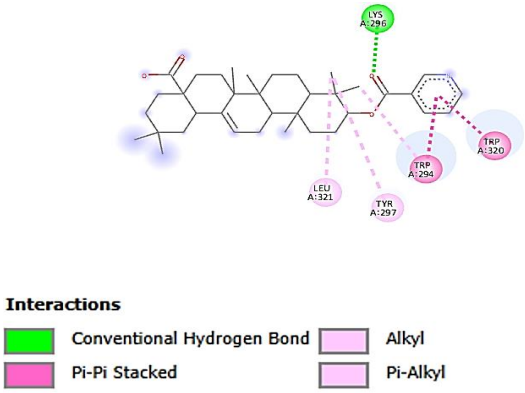


Figure 2(f): Interaction of α-amylase (AAM-1B2Y) with NA

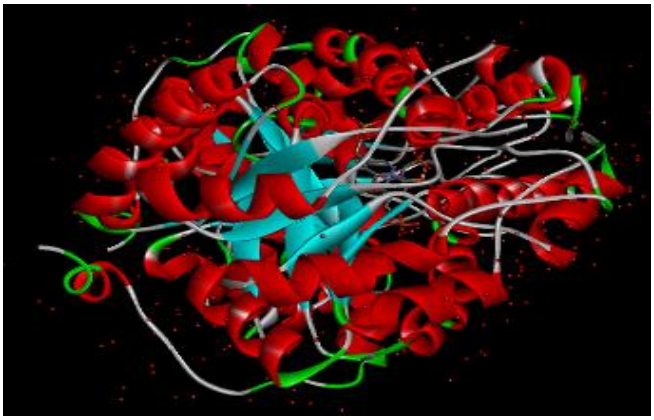


Figure 2 (g): Fructose-1,6-diphosphatase, F1,6DP (3OHI)

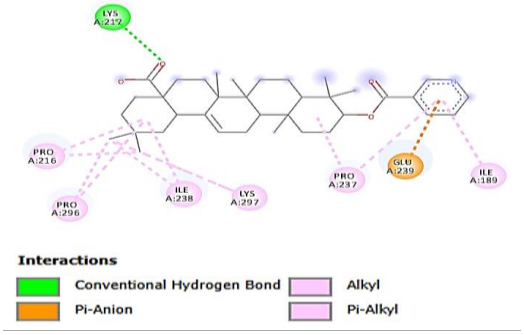


Figure 2(h): Interaction of F1,6DP (3OHI) with ND

Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.13 (1): 65-75; March 2024 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v13-i1-535

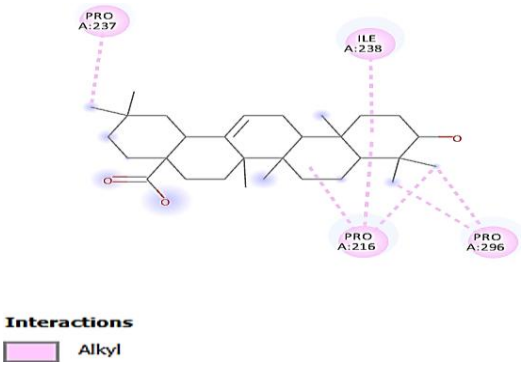


Figure 2(i): Interaction of F1,6DP (3OHI) with OA

Figure 2 (j): Dipeptidyl Peptidase-4; DPP-4 (IJ2E)

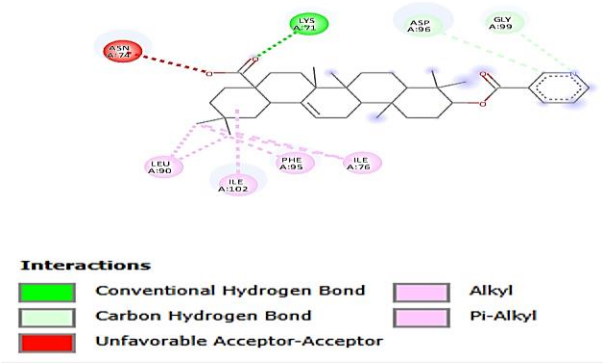


Figure 2 (k): Interaction of DPP-4 (IJ2E) with ND

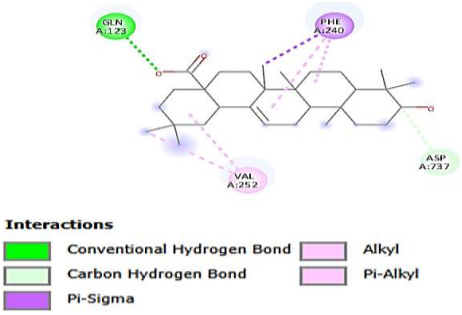


Figure 2 (l): Interaction of DPP-4 (IJ2E) with

3.3 Result of physicochemical properties of OA and ND

The Result of solubility profile and other physicochemical properties of OA and ND are presented in tables 4 and 5.

Table 4: Result of Solubility Profile of OA and ND

Ligand	*Solvents*							
	Water	*CH_2Cl_2*	*$CHCl_3$*	*Absolute Ethanol*	*n-Hexane*	*Diethyl ether*	*Acetone*	*Methanol*
OA	-	++	++	+	+	+	++	+
ND	-	++	++	++	+	+	++	++

(-)-insoluble; (+)-slightly soluble; (++)-soluble

Table 5: Physicochemical properties of OA and ND

Ligand	*Physical Appearance*	*Melting point (°C)*	*Amax /Abs*	*Rf-value: n-hexane-ethylacetate (3:2)*
OA	*White powder*	*306-310*	*349.5 nm at 0.6405 A*	*0.28*
ND	*Creamy powder*	*330-335*	*359.5 nm at 1.2216 A*	*0.37*

3.3 Result of in vitro studies

Result of *in vitro* antidiabetic studies are presented in tables 7 and 8 while graphical representations are in figures 3 and 4.

Table 6: Percentage (%) Inhibition of α-Amylase by OA, ND and Acarbose

Conc. (µg/mL)	OA (%)	OA IC_{50}	ND (%)	ND IC_{50}	Acarbose (%)	Acarbose IC_{50}
20	46.15±6.80		50.20±4.30		30.15±5.50	
40	52.21±4.50		56.12±5.40	24.25±0.52	45.20±4.30	
60	70.40±6.20	26.40±0.32	73.42±6.50		62.42±5.40	48.21±0.56
80	74.62±5.40		78.45±5.70		70.61±6.40	
100	83.10±5.20		90.24±6.70		76.34±6.80	

Data are given as mean±SEM (n = 2; p < 0.05)

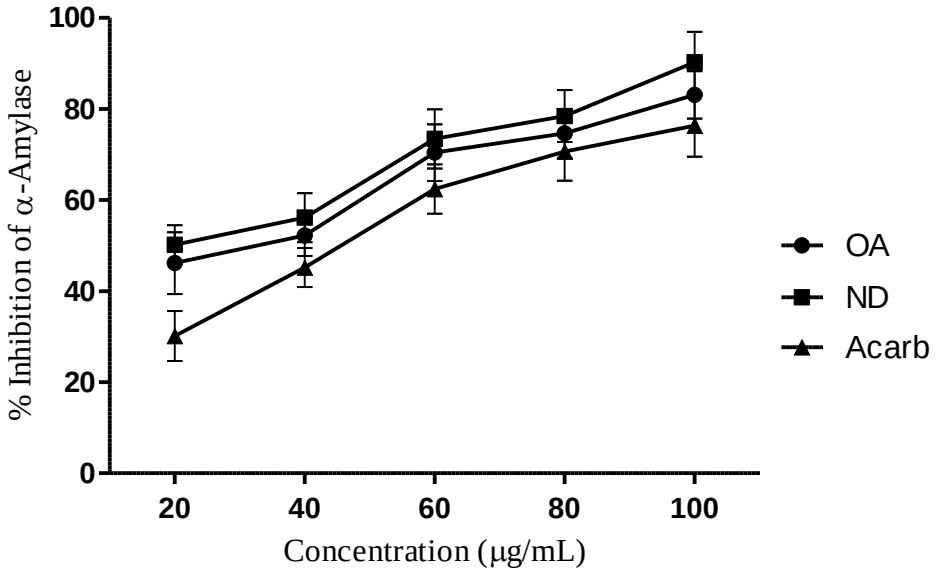


Figure 3: Graph of % Inhibition of α-Amylase by OA, ND and Acarb (Acarbose)

Table 7: Percentage increase in Glucose Uptake by yeast cell for OA, ND and Glibenclamide

Ligand	Percentage (%) Increase				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
OA	50.10±6.50	58.00±4.50	65.10±4.30	72.20±5.40	80.20±7.20
ND	56.20±4.30	60.50±5.40	68.60±6.50	77.70±5.70	88.50±4.70
Glibenclamide	40.10±5.50	51.20±4.30	60.40±5.40	67.20±6.20	72.60±6.80

Data are given as mean±SEM (n = 2; p < 0.05)

Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.13 (1): 65-75; March 2024 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v13-i1-535

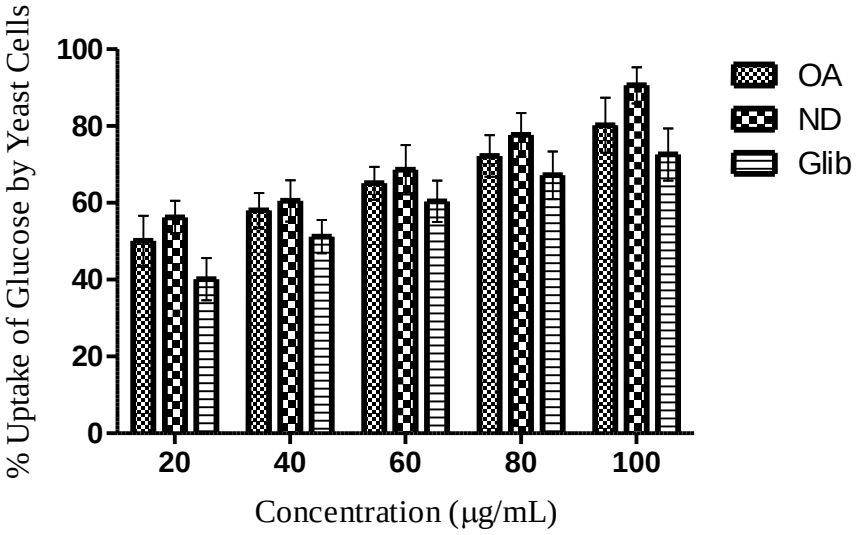


Figure 4: Percentage increase in Glucose Uptake by yeast cell for OA, ND and Glib (Glibenclamide)

Important findings:
In silico studies shows ND showing good binding affinities over OA at the following targets; GLK (OA = -8.5; ND = -9.1); AAM (OA = -8.0; ND = -9.0); PPARG (OA = -7.4; ND = -9.0); DPP4 (OA = -8.4; ND = -9.3); GSK3 (OA = -8.3; ND = -8.9); F16DP (OA = -7.6; ND = -8.8) and PTB1B (OA = -8.6; ND = -9.3) For the alpha-amylase inhibition, ND shows maximal % inhibition and lower IC_{50} (OA = 83.3; 26.40±0.32, ND = 90.24;24.25±0.52, Acarbose = 76.34; 48.21±0.56). For the result of % glucose uptake by yeast cell, ND values were higher (OA = 80.2, ND = 88.5, Glibenclamide = 72.6). Both the *in silico* and i*n vitro* results agree together that ND has a better prospect as antidiabetic drug than all the compounds tested together.

4. DISCUSSION

The reaction mechanisms for the formation of nicotinoyl chloride and 3-oleanyl niacinate are postulated in figures 5 and 6 respectively.

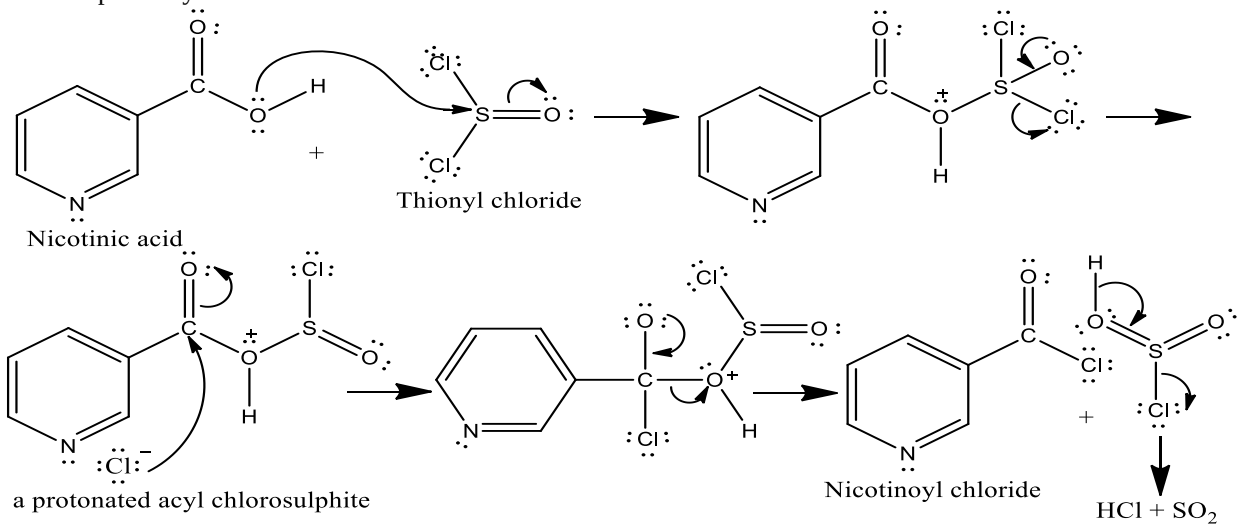


Figure 5: Mechanism of formation of nicotinoyl chloride

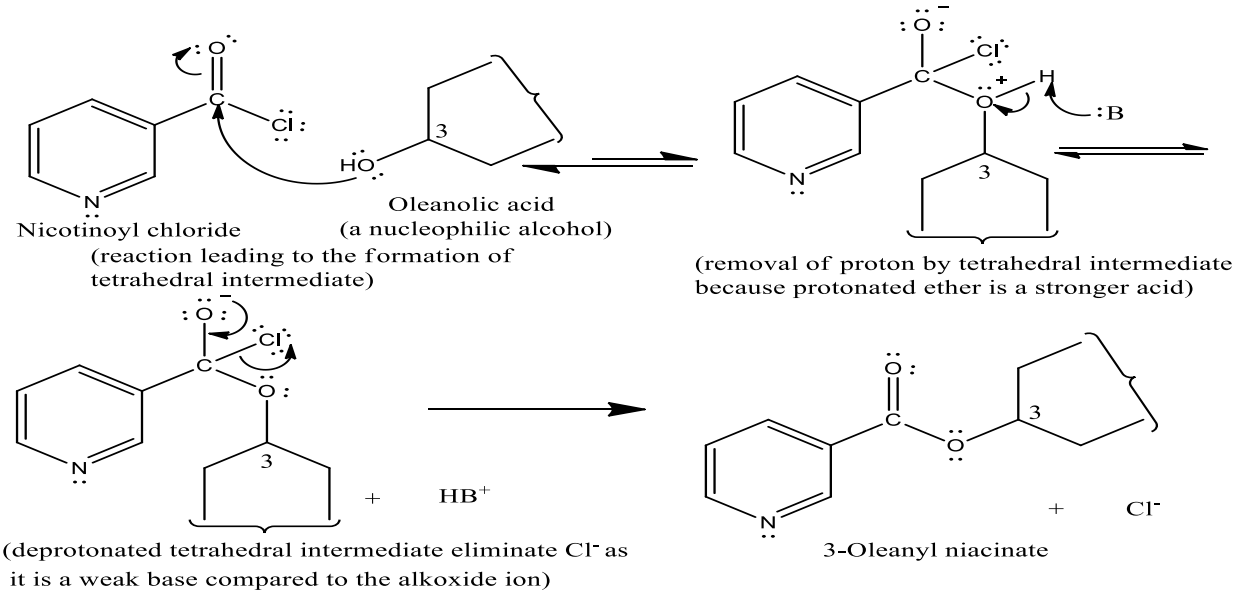


Figure 6: Mechanism of base-catalyzed formation of 3-Oleanyl niacinate

The distinct Rf-value and physicochemical properties of the new compound showed that the compound is 3-oleanyl niacinate and different from OA. The findings of Liu; Pollier and Goossens [4,5] on how OA exerts beneficial effects against diabetes and metabolic syndrome is further confirmed in this research. In all the test procedures carried out, OA was tested alongside and showed remarkable antidiabetic activity over control compounds. However, niacin ester derivative showed higher activity. This is confirmed in its *in silico* assay on seven protein targets and in the *in vitro* assay. The higher activity of OA over ND can be due to the extra active binding sites of amino acids on ND over OA [figure 2(a)-(l)]. This claim of OA and derivatives possessing remarkable antidiabetic potency is confirmed by other researchers [17].

5. CONCLUSION

In all area of testing, Niacin derivative of oleanolic acid (ND) showed high prospect as drug for treatment of Type 2 diabetes mellitus. Other oleanolic acid derivatives such as cinnamic acid, gallic acid and benzoic acid derivatives that equally showed good binding affinities will likely possess good antidiabetic potency following their further study.

Acknowledgment

The authors wish to thank Mrs. Ekaette Umoh (Chief Technologist) of the University of Uyo for her immense contribution towards the success of this work. This work was supported by a grant from TETFunD (IBR), Nigeria.

Conflict of Interest

There is no conflict of interest.

Contribution of the Authors

VUA anchored the entire work. SJO, RAU and NJO partook in the *in vitro* studies. ASE took part in the analysis while OAE handled *in silico* studies.

REFERENCES

[1] WHO (2019). Classification of Diabetes mellitus, 2019. https://www.who.int/health-topics/diabetes. Retrieved 19-06-2023.

[2] Al-Khafajy, D. A., Majeed,M. J., Al-Azzawi, O. F. and Khaleel, A. I. (2021). Role of CoQ10 and IGFBP-1 in Obesse male patients with DM Type II. Indian *Journal Forensic Med. Toxicol*, 15:2205 [Google Scholar] [Cross Ref].

[3] Lankatillake, C, Huynh, T. and Dias, D, A. (2019). Understanding Glycaemic Control and Current Approaches for Screening Antidiabetic Natural Products from Evidence-based Medicinal Plants, *Plant Methods*. 15:105-140.

[4] Liu, J. (1995). Pharmacology of Oleanolic acid and Ursolic acid. *Journal of Ethnopharmacology*, 49(2):57–68.

[5] Pollier, J. and Goossens, A. (2012). Oleanolic Acid. *Phytochemistry*, 77:10-20.

[6] Castelano J. M., Guinda, A., Delgado, T., Rada, M. and Cayuela, J. A. (2013). Biochemical Basis of the anti-diabetic activity of Oleanolic acid and Related Pentacyclic Triterpenes. *Diabetes*. 62(6):1791-1799.

[7] Guedes, I. A., Costa, L. S. C., Dardenne, L. E. (2021). Drug Design and repurposing with Dockthor-Vs web Server focusing on SAR-Cov-2 therapeutic targets and their non-synonym variants. *Scientific Report,* 11:5543-51.

[8] Guo,T., Wu, S., Guo, S., Bai, L., Liu, Q and Bai, N. (2015). Synthesis and Evaluation of a series of oleanolic acid saponins as alpha-glucosidase and alpha-amylase inhibitors, *Arch Pharm Chem Life Sciences*, 348: 615-628.

[9] Gomez, Y. R., Paoli, P., Navarrete-Vazquez, G., Estrado-Soto, S., Ramirez-Espinosa, J. J., Rioss, M. Y., Flores-Morales, V., Camici, G., Rosa-Lugo, V. and Hidalgo-Figueroa, S. (2014). Synthesis of oleanolic acid derivatives: *In vitro*, *in vivo* and *in silico* studies for PTP-1B Inhibition, *European Journal of Medicinal Chemistry*, 87:316-327.

[10] Fadipe, V.O., Opoku, A. R. Shintre, S. A. Singh, P., M. Singh, M and Mongalo, N. I. (2016). Antimycobacterial, antiplasmodial and toxicological effect of oleanolic acid and its derivative from *Syzygium aromaticum* Linn (Myrtaceae). *South African Journal of Botany*,100(103):314-330.

[11] Bednarczk-Cwynar and B., Zaprutko, L. (2015). Recent Advances in Synthesis and Biological activity of triterpenoic acid acylated oximes. *Phytochem. Rev*, 14:203-231.

[12] Eseyin, O.A., Johnson, E. C., Etim, E. I., Igboasoiyi, A. C., Attih, E. E., Udobre, A. S., Ebong, A. S., Anthony, P. C., Asanga, E. E., Charles, G. E., and Daniel, A. O. (2022). *In silico* evaluation of the antidiabetic potentials of some quercetin derivatives. *Journal of Drug Discovery and Research* 1(1): 1-15.

[13] Trott, O. and Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of Computational Chemistry*, 31: 455-461.

[14] Morrison, R. T. and Boyd, R. N. (2007). *Organic Chemistry*. 6th Ed, Pearson Education Ltd., London, pp798.

[15] Hansawasdi, C., Kawabata, J. and Takanori, K. (2000). Alpha-Amylase Inhibitors from Roselle (*Hibiscus sabdariffa* Linn.) Tea. *Bioscience, Biotechnology and Biochemistry*, 64:1041-1043.

[16] Cirillo, V. (2008). Sugar Transport in Psychrophilic Yeast. *Journal of Bacteriology*, 106:247-252.

[17] Johnson, E.C., Ilyas, M., Eseyin, O.A., Etim, E.I., Udobre, A.S., Udoh, A.E., and Edem, E.O. (2016). Isolation, characterization and anti-diabetic potentials of oleanolic acid from the leaves of *Aspilia africana* (Pers) C.D Adams (Asteraceae). *Journal of Pharmaceutical Sciences*, 2(2), 62-65